

[0066] The breeding values of the bulls are centrally estimated by the United Information Systems Animal Production (Vereinigte Informationssysteme Tierhaltung—VIT) in Verden. A total amount of more than 150,000 daughters and their performance data are integrated in the estimation of the breeding values. From all bulls, deregressed breeding values, concerning the milk yield, the protein and fat yield, the protein content (in %) and the fat content (in %), are utilized in the variance component estimation. The deregression of the breeding values is carried out as described by Thomsen et al. (2001, *J Anim Breed Genet.* 118, 357-370).

[0067] The variance component estimation is carried out using the program package SAS. First, as unique fixed effect, the marker CSN1S1 is considered in the model, because other influence factors (e.g. operational effects, milking frequency) are already corrected in the frame of the estimation of the breeding value and the deregression (influence of the sires). The analysis reveals significant effects of the marker CSN1S1 on all studied traits (deregressed breeding values for protein percentage (DRG_PP), milk yield (DRG_MY1), fat yield (DRG_FY1), protein yield (DRG_PY1), fat percentage (DRG_FP)). Table 3 shows the effect of CSN1S1 on deregressed breeding values for milk production traits, indicating also the probability of error (p) for the effects on the individual traits.

TABLE 3

Trait	Probability of error (p)
DRG_PP	<0.0001
DRG_MY1	0.0011
DRG_FY1	0.0016
DRG_PY1	0.0056
DRG_FP	0.0052

[0068] The highest significance is calculated for the effect on DRG_PP. As the examined marker CSN1S1 is located directly within the regulatory region of a milk protein gene,

this could be an indication of a direct effect. The marker CSN1S1 fulfils the requirements to a functional candidate gene.

[0069] The highest breeding value for milk (DRG_MY1) is achieved on average by bulls with the genotype 12, whereas the highest breeding values for protein percentage (DRG_PP) are found within the group with genotype 24. Table 4 shows a compilation of the least square means (LS_means) for the groups with the genotypes 12, 22, 23 and 24. The table displays the LS_means as well as standard errors for the deregressed breeding values for milk yield (DRG_MY1) and protein percentage (DRG_PP) in groups with different CSN1S1 genotypes.

TABLE 4

CSN1S1 type	n	LSMEAN ± se	
		DRG_MY1	DRG_PP
12	79	198.232 ± 15.700	-0.00022534 ± 0.00006470
22	398	155.341 ± 6.995	-0.00037495 ± 0.00002921
23	131	138.806 ± 12.192	-0.00038405 ± 0.00005271
24	76	112.364 ± 16.007	0.00008175 ± 0.00006650
Alle	684	152.353	-0.000307

[0070] In order to obtain a more exact clarification, the variance analysis is repeated within individual families and groups of families with identical genotypes. Hereby is revealed, that the effect on the milk yield can not be confirmed in all families. In family 9, in which the sires exclusively passed down the allele 2, the only remaining effect is encountered close to the 5% threshold of significance for DRG_PP (p=0.0610). Furthermore, a comparison of the LS_means for the traits DRG_MY1, DRG_PP, DRG_FP is carried out for all groups of genotypes and within each individual family, and it is proved whether the difference of the LS-means between the genotypes 12, 23 and 24 and the most frequent genotype 22 is significant. The results are graphically illustrated in FIG. 5.

SEQUENCE LISTING

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<220> FEATURE:

<221> NAME/KEY: Primer 2

<222> LOCATION: (1)..(18)

<223> OTHER INFORMATION: 18 basepair, single stranded nucleic acid (linear)

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<223> OTHER INFORMATION: 19 basepair, single stranded nucleic acid
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<212> TYPE: DNA
<213> ORGANISM: Bos taurus
<220> FEATURE:
<221> NAME/KEY: alpha-S1Kaseingen
<222> LOCATION: (1)..(1061)
<223> OTHER INFORMATION: start Exon 1 at position 620
<300> PUBLICATION INFORMATION:
<301> AUTHORS: Koczan Dirk, Hobom Gerd, Seyfert Hans-Martin
<302> TITLE: Genomic organization of the bovine alpha S1-casein gene
<303> JOURNAL: Nucleic acids research
<304> VOLUME: 19
<305> ISSUE: 20
<306> PAGES: 5591
<307> DATE: 1991-09-24
<308> DATABASE ACCESSION NUMBER: X59856
<309> DATABASE ENTRY DATE: 1991-07-18
<313> RELEVANT RESIDUES: (1)..(1061)
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: EMBL X59856
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gattagacca catataatgt aacttatttc acaaggtaaa taattataat aaataatatg      180
gattaactga gttttaaaag gtgaaataaa taatgaattc ttctcatgtt ctgtatgtt      240
aataaaaaattt gaaaaattttt gaagacccca ttttgccca agaatttcat ttacaggat      300
tgaatttttc aaaggttaca aaggaaattt tattgatata ataaatgcat gttctataa      360
taaccataaa tctagggtt tgggggtt ttttttgtt tggtaattta gaacaatgcc      420
atccatattc ctgtataatg agtcaattct ttgttgtaaa ctctccttag aatttcttgg      480
gagaggaact gaacagaaca ttgatttcct atgtgagaga attcttagaa tttaataaaa      540
cctgttgggtt aactgaaac cacaaaaatc gcattttact aatcgttggg tttaatagc      600
ttggaaagcaa aagtctgcac tccacccatgat catcaacccca gcttgcgtt tttcccaat      660
cttgggttca aggttattatg tatacatata acaaaaaatttc tatgattttc ctctgttca      720
tctttcatc ttcaactata cgcaaggtaa acttttctat gtgattgcaat gtattggat      780
tttcctatgtataactgttta gcttaaaaat atatgttcaat atgttgcatac tatctatctc      840
agagctatacgtgaaaaattt aatatactttt ataaagacca aattgtatcat tttaaacgaa      900
aatttctataat tactgaaaat gtatgtatcat aacttcctgtat tagattttatgtt gtaaaaataat      960
ttgaatcatt ttgtcaat tctgtaaaaat gttgtcatac agaataatattt aatataatttt      1020
tgggggttcaat gatataacat ttctggtaga atatgttcaag g                                1061

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  Exon 1 at position 617
<222> LOCATION: (1)...(652)
<223> OTHER INFORMATION: Mutation/SNP position 83 (A to G), position 98
  (A to G), position 298 (A to C), position 442 (A to G; change/loss
  of YY1- and AP1 -bindingsite), position 541 (G to A);
  deletion TT between position 389 and 394 compaired with Allel2
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gattagacca catataatgt aacttatttc acaaggtaaa taattataat aaataatatg    180
gattaactga gttttaaaag gtgaaataaa taatgaatcc ttctcatggc cttgtatgtt    240
aataaaaattt gaaaaattttt gaagacccca ttttgccca aaaaaaaaaa ttacaggat    300
tgaatttttc aaaggttaca aaggaaattt tattgtatata aaaaaaaaaa gttctcataa    360
taaccataaa tctagggtt tgggggggtt tttttttttt taatttagaa aatggccatt    420
ccatttcctg tataatgagt cgcttcattt tttttttttt ttctttagaa ttcttggag    480
aggaactgaa cagaacatgt atttcctatg tgagagaattt cttttttttt aaaaaaaaaa    540
atgggttaaa ctgaaaccac aaaaaaaaaa ttttttttttt cttttttttt aaaaaaaaaa    600
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<222> LOCATION: (1)...(654)
<223> OTHER INFORMATION: Bindingsite for transcriptionfactor AP-1 at
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  position 443 to 448
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gattaactga gttttaaaag gtgaaataaa taatgaatcc ttctcatggc cttgtatgtt    240
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tgaatttttc aaaggttaca aaggaaattt tattgtatata aaaaaaaaaa gttctcataa    360
taaccataaa tctagggtt tgggggggtt tttttttttt tttaattttttt aatggccatt    420
ttccatttcc tttttttttt ttccatttcc tttttttttt tttaattttttt aatggccatt    480
agaggaactgaa cagaacatgt ttttttttttt ttttttttttt ttttttttttt ttttttttttt    540
ctgttggta aactgaaaccac aaaaaaaaaa ttttttttttt ttttttttttt ttttttttttt    600
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<210> SEQ ID NO 6

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<222> LOCATION: (1)..(650)
<223> OTHER INFORMATION: Bindingsite for transcriptionfactor AP-1 at
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    position 439 to 444 deletion G and TTT between 390 and 396
    compaired with Allel 2

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gtataattaa aatgccacca aaatttatac aataattata ttttctttt gcagggaaaa	120
gattagacca catataatgt aacttatttc acaaggtaaa taattataat aaataatag	180
gattaactga gttttaaaag gtgaaataaa taatgaatcc ttctcatggc ttgtatgtt	240
aataaaaatt gaaaaatttt gaagacccca ttttgcacca agaatttcat ttacaggat	300
tgaattttc aaaggttaca aaggaaattt tattgtata ataaatgcat gttctcataa	360
taaccataaa tctagggttt tgggggttt tttttgtta atttagaaca atgccccatcc	420
atttcctgtt taatgagtca cttctttgtt gttaactctc ctttagaattt cttggggag	480
gaactgaaca gaacattgtt ttcctatgtt agagaattct tagaatttaa ataaacctgt	540
tggtaaact gaaaccacaa aatttagcatt ttactaatca gtaggtttaa atagcttgg	600
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<222> LOCATION: (1)..(650)
<223> OTHER INFORMATION: Bindingsite for transcriptionfactors: AP-1 at
    position 434 to 441, ABF1 at position 469 to 483, YY-1 at
    position 439 to 444; mutation (SNP) at position 480 (G to C),
    developing a ABF1-bindingsite;
    deletion G and TTT between position 390 and 396 compaired with Al
    lel 2

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gaatgaatga actagttacc acaaactagta cacccaaaat gaacaaaaaa tagcttggtg	60
gtataattaa aatgccacca aaatttatac aataattata ttttctttt gcagggaaaa	120
gattagacca catataatgt aacttatttc acaaggtaaa taattataat aaataatag	180
gattaactga gttttaaaag gtgaaataaa taatgaatcc ttctcatggc ttgtatgtt	240
aataaaaatt gaaaaatttt gaagacccca ttttgcacca agaatttcat ttacaggat	300
tgaattttc aaaggttaca aaggaaattt tattgtata ataaatgcat gttctcataa	360
taaccataaa tctagggttt tgggggttt tttttgtta atttagaaca atgccccatcc	420
atttcctgtt taatgagtca cttctttgtt gttaactctc ctttagaattt cttggggag	480
gaactgaaca gaacattgtt ttcctatgtt agagaattct tagaatttaa ataaacctgt	540
tggtaaact gaaaccacaa aatttagcatt ttactaatca gtaggtttaa atagcttgg	600
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<221> NAME/KEY: Primer1
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: 20 basepair, single stranded nucleic acid
    (linear)

<400> SEQUENCE: 8

gaatgaatga actagttacc

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1. Genetic marker at the 5'-flanking region of the α S1 casein gene (CSN1S1) characterized by the fact that it contains the nucleotide sequence 1-1061, preferably the nucleotide sequence 1-655 at the 5'-flanking region of the α S1 casein gene.

2. Genetic marker according to patent claim 1 characterized by its amplification by means of PCR reaction either through

Primer 1
CSN1S1pro1f (5' GAA TGA ATG AAC TAG TTA CC 3')

Primer 2
CSN1S1pro1r (5' GAA GAA GCA GCA AGC TGG 3')

or through

Primer 1
CSN1S1pro1f (5' GAA TGA ATG AAC TAG TTA CC 3')

Primer 3
CSN1S1pro2r (5' CCT TGA AAT ATT CTA CCA G 3')

3. Genetic marker according to patent claim 1 characterized by its variability within milk breeds.

4. Genetic marker according to patent claim 1 characterized by its utilization in order to determine the allelic state at the 5'-flanking region of the α S1 casein gene.

5. Procedure to determine the allelic state of the 5'-flanking region of the α S1 casein gene, characterized by the following steps:

a) provision of the source material of the organism to be examined

b) isolation of the genetic material

c) targeted isolation or enrichment of the marker fragment at the 5' region of the α S1 casein gene or of a sequence, which contains portions of the marker sequence, preferably the fragment 1 to 655 of the marker sequence out of the α S1 casein gene

d) Proof of the allelic state in the isolated or enriched sequence fragment of the marker fragment of the α S1 casein gene.

6. Procedure according to patent claim 5 characterized by the utilization of source material coming from an animal, particularly a mammal, in particular a bovine, a sheep or a goat, including breed animals and embryos of these species.

7. Procedure according to patent claim 5 characterized by the utilization of blood, leukocytes, tissue including biopsy material, milk, sperm, hair, individual cells including cell material from embryos, a bacteria culture or isolated chromosomes as source material.

8. Procedure according to patent claim 5 characterized by the utilization of source material coming from a genetically modified organism (GMO) which contains the marker fragment of the α S1 casein gene.

9. Procedure according to patent claim 5 characterized by the utilization of genetic material containing genomic DNA or RNA from animals, plasmid DNA from bacteria, from artificial chromosomes such as BACs and YACs.

10. Procedure according to patent claim 5 characterized by achieving the enrichment of the marker segment of the α S1 casein gene by means of polymerase chain-reaction.

11. Procedure according to patent claim 5 characterized by the enrichment of the marker segment of the α S1 casein gene by means of polymerase chain-reaction with the oligonucleotides

Primer 1
CSN1S1pro1f (5' GAA TGA ATG AAC TAG TTA CC 3')

Primer 2
CSN1S1pro1r (5' GAA GAA GCA GCA AGC TGG 3')

Primer 3
CSN1S1pro2r (5' CCT TGA AAT ATT CTA CCA G 3')

as primers, whereby the following combinations are selected: primer 1 with primer 2 and primer 2 with primer 3.

12. Procedure according to patent claim 5 characterized by the determination the allelic state by means of SSCP, RFLP, OLA, TGGE, ASPCR, PCR-ELISA, microarray method or through nucleic acid sequencing.

13. Procedure according to patent claim 5 characterized by detection of one or more of the allelic states of the marker sequence of the α S1 casein gene.

14. Utilization of the procedure according to claim 5 in order to examine the animals' milk production traits, independently of age and lactation.

15. Utilization of the procedure according to claim 5 in order to select organisms which carry a certain allelic state or a certain genotype of the marker sequence of the α S1 casein gene or a portion thereof.

16. Utilization of the procedure according to claim 5 in breeding programs, particularly for a marker-supported selection.